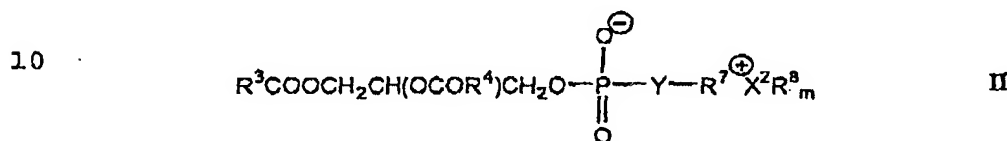


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CLAIMS

1. An oral vaccine comprising a nucleic acid operatively encoding an antigen complexed with or entrapped within liposomes formed from liposome forming components including cationic compounds and zwitterionic phospholipid in molar ratio in the range 1:1 to 1:5, and comprising
- 5 a) at least one cationic compound
- b) at least 50% by mole of the zwitterionic phospholipid has the general formula II



in which R^3 and R^4 are the same or different and are selected from groups of the formula $\text{CH}_3(\text{CH}_2)_e(\text{CH}_2)_g-$

- 15 in which each of e and g are 0 to 23 and $e + g$ is in the range 12 to 23;

R^7 is a C_{1-8} alkanediyl group;

Y is $-\text{O}-$ or a bond;

X^2 is N, P or S;

- 20 m is 3 when X^2 is N or P and is 2 when X^2 is S; and

the groups R^8 are the same or different and are selected from the group consisting of hydrogen, C_{1-8} alkyl, C_{6-11} aryl or aralkyl, or two or three of the groups R^8 together with X^3 may form a saturated or unsaturated heterocyclic group having 5 to 7 ring atoms.

- 25 2. A vaccine according to claim 1 in which the cationic compound has the general formula I,



in which R^1 and R^2 are the same or different and are selected from groups of the formula $\text{CH}_3(\text{CH}_2)_a(\text{CH}=\text{CH}-\text{CH}_2)_b(\text{CH}_2)_c(\text{CO})_d-$

- 30 in which b is 0 to 6, a and c are each selected from 0-23 and $(a + c + 3b)$ is in the range 12-23 and d is 0 or 1;

R^5 is a bond or a C_{1-8} alkanediyl group;

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X^1 is N, P or S;

n is 3 where X^1 is N or P and is 2 where X^1 is S; and

the groups R^8 are the same or different and are selected from

hydrogen, C_{1-8} alkyl, C_{6-12} aryl or aralkyl, or two or three of the groups R^8

5 together with X^1 may form a saturated or unsaturated heterocyclic group having 5 to 7 ring atoms.

3. A vaccine according to claim 2 in which $R^1=R^2$ and $R^3=R^4$.

4. A vaccine according to claim 3 in which R^1 and R^2 represent a different group to R^3 and R^4 .

10 5. A vaccine according to claim 3 and claim 4 in which in R^1 and R^2 $b=1$ and in which $(a + c)$ is in the range 10-20.

6. A vaccine according to any of claims 3 to 5 in which $d = 0$.

7. A vaccine according to any of claims 2 to 6 in which X^1 is N and in which the R^8 groups are all C_{1-4} alkyl.

15 8. A vaccine according to any preceding claim which comprises two zwitterionic phospholipids each having the formula II, in which Y is O, and X^2 is N, and the groups R^8 of the first phospholipid are all hydrogen and the groups R^8 of the second phospholipid are all C_{1-4} alkyl, preferably methyl.

20 9. A vaccine according to claim 8 in which, in each phospholipid Y is O and R^7 is $(CH_2)_h$ in which h is 2 or 3.

10. A vaccine according to claim 8 or claim 9 in which the groups R^3 and R^4 of the first phospholipid are the same and each is a group in which $f=1$ and $(e + g)$ is in the range 10 to 20, preferably 12 to 14.

25 11. A vaccine according to any of claims 8 to 10 in which the groups R^3 and R^4 of the second phospholipid are the same and $e + g$ is in the range 15 to 23, preferably 15-17.

12. A vaccine according to any of claims 12 to 14 in which the zwitterionic phospholipid is selected from the group consisting of
30 distearoylphosphatidylcholine, distearoylphosphatidylethanolamine, dipalmitoylphosphatidylcholine, dipalmitoylphosphatidylethanolamine and mixtures thereof.

13. A vaccine according to claim 1 in which the cationic compound is cholesterol-3 β -N-(dimethylaminoethyl) carbamate.

14. An oral vaccine according to any preceding claim in which the liposome forming components include at least 25 mole%, preferably at least
5 50 mole%, of components which individually have a transition temperature of more than 40°C.

15. A vaccine according to any preceding claim in which the nucleic acid is entrapped within the liposomes.

16. A method in which a human or a non-human animal is
10 vaccinated by administering a vaccine according to any preceding claim orally whereby an immune response to the encoded antigen is generated.

17. A method of entrapping polynucleotide into liposomes involving the steps of:

15 i) forming an aqueous suspension comprising naked nucleic acid, which operatively encodes an immunogenic polypeptide useful to induce a desired immune response in a human or animal subject, and preformed liposomes formed of liposome forming components as defined in any of claims 1 to 14,

ii) freeze-drying or spray-drying the suspension, and

20 iii) rehydrating the product of step ii) to form dehydration/rehydration vesicles.

18. A method according to claim 17 comprising the further steps of:

iv) subjecting the aqueous suspension of dehydration/rehydration vesicles from step iii) to microfluidization to control their size;
25 and

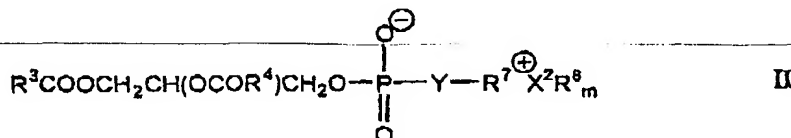
v) optionally separating non entrapped nucleic acid from liposomes.

19. Use of a nucleic acid operatively encoding an antigen
complexed with or entrapped within liposomes formed from liposome forming
30 components including cationic compounds and zwitterionic phospholipid in molar ratio in the range 1:1 to 1:5, and comprising

a) at least one cationic compound

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b) at least 50% by mole of the zwitterionic phospholipid has the general formula II



in which R^3 and R^4 are the same or different and are selected from groups of the formula $\text{CH}_3(\text{CH}_2)_e(\text{CH}_2)_g-$

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R^7 is a C_{1-8} alkanediyl group;

Y is $-\text{O}-$ or a bond;

X^2 is N, P or S;

m is 3 when X^2 is N or P and is 2 when X^2 is S; and

15 the groups R^8 are the same or different and are selected from the group consisting of hydrogen, C_{1-8} alkyl, C_{6-11} aryl or aralkyl, or two or three of the groups R^8 together with X^3 may form a saturated or unsaturated heterocyclic group having 5 to 7 ring atoms;

20 in the manufacture of an oral vaccine for use in the vaccination of an animal in a method in which the vaccine is administered orally.

20. Use according to claim 19 in which the vaccine is as claimed in any of claims 2 to 15.

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